

Elimination of *Escherichia coli* O157:H7 in Meats by Gamma Irradiation

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Undercooked and raw meat has been linked to outbreaks of hemorrhagic diarrhea due to the presence of *Escherichia coli* O157:H7; therefore, treatment with ionizing radiation was investigated as a potential method for the elimination of this organism. Response-surface methods were used to study the effects of irradiation dose (0 to 2.0 kGy), temperature (−20 to +20°C), and atmosphere (air and vacuum) on *E. coli* O157:H7 in mechanically deboned chicken meat. Differences in irradiation dose and temperature significantly affected the results. Ninety percent of the viable *E. coli* in chicken meat was eliminated by doses of 0.27 kGy at +5°C and 0.42 kGy at −5°C. Small, but significant, differences in radiation resistance by *E. coli* were found when finely ground lean beef rather than chicken was the substrate. Unlike nonirradiated samples, no measurable verotoxin was found in finely ground lean beef which had been inoculated with $10^{4.8}$ CFU of *E. coli* O157:H7 per g, irradiated at a minimum dose of 1.5 kGy, and temperature abused at 35°C for 20 h. Irradiation is an effective method to control this food-borne pathogen.

Escherichia coli O157:H7 is a rapidly emerging food-borne pathogen that can produce a clinical illness characterized by an acute grossly bloody diarrhea that is accompanied by severe, crampy abdominal pain (2, 13, 24). A few patients go on to develop hemolytic uremic syndrome or thrombotic thrombocytopenic purpura (2, 13). This pathogen has been associated primarily with undercooked and raw beef, lamb, pork, or poultry (2, 5, 13, 17). The recent approval of ionizing radiation treatments of poultry to eliminate food-borne pathogens (1, 3) makes it appropriate to determine the effect that irradiation treatments would have on this pathogen in poultry. The regulation for irradiation of poultry products from the USDA Food Safety and Inspection Service requires minimum and maximum doses of 1.5 and 3.0 kGy (1 kGy = 100 kilorads), respectively (3). Though beef is not included under the current regulation, it seemed prudent to include it in the study, since several of the outbreaks of the disease have been specifically linked to beef (2, 13) and raw beef is consumed as steak tartare. No previous studies of the effects of ionizing radiation on *E. coli* O157:H7 were found in the literature. The aims of this study were to determine the sensitivity of *E. coli* O157:H7 suspended in beef or mechanically deboned chicken meat (MDCM) to gamma radiation and also to determine the influence of processing parameters such as atmosphere or temperature on that sensitivity.

(Portions of this paper were presented at the 92nd General Meeting of the American Society for Microbiology, New Orleans, La., 26 to 30 May 1992.)

MATERIALS AND METHODS

Organisms. *E. coli* O157:H7 ATCC 43895 and *E. coli* ATCC 25922 were maintained and cloned on tryptic soy agar (Difco, Detroit, Mich.) and incubated at 35°C. *E. coli* O157:H7 ATCC 43895 was implicated in a hemorrhagic colitis outbreak involving raw hamburger meat and produces Shiga-like toxins I and II (24). *E. coli* ATCC 25922 is a

nonpathogenic clinical isolate. One milliliter from a 15- to 18-h culture of the appropriate strain incubated at 35°C in Trypticase soy broth (BBL, Cockeysville, Md.) was used to inoculate 100 ml of Trypticase soy broth in a 500-ml baffled shake flask. These cultures were incubated aerobically with shaking (150 rpm) at 35°C and harvested at 16 h for stationary-phase cells and at 4 h for mid-log-phase cells. A 10-fold cell concentrate was prepared for many studies by centrifuging the cells and resuspending them in 1/10 volume of Butterfield's phosphate (0.25 M KH_2PO_4 adjusted to pH 7.2 with NaOH).

Substrates. MDCM was obtained from a commercial manufacturer of poultry frankfurters. The mean proximate analysis of three separate lots of MDCM was 21.3% fat, 65.6% moisture, 14.0% protein, and 0.95% ash. Triple-ground lean top round beef, such as would be used in the preparation of steak tartare, was obtained from a local butcher shop. The proximate analysis for the ground beef was 2.6% fat, 73.5% moisture, 19.8% protein, and 1.4% ash. The MDCM and the ground beef were subdivided into 100 ± 0.05 -g amounts and then spread thinly and vacuum sealed in Stomacher 400 (Tekmar Co., Cincinnati, Ohio) polyethylene bags. These bags were themselves vacuum sealed in Freshstuff (American National Can Company, Des Moines, Iowa) oxygen barrier pouches (oxygen transmission, 0.6 to 0.8 $\text{cm}^3/645 \text{ cm}^2/24 \text{ h}$ at 3.2°C and 90% relative humidity). These meats were sterilized by gamma irradiation at a dose of 42 kGy at −50°C. Both sterile and nonsterile MDCM and ground beef were stored at −20°C until use.

Irradiation. The self-contained gamma radiation source was ^{137}Cs with a strength of approximately 134,000 Ci and a dose rate of 0.12 kGy min^{-1} . The dose rate was established by using National Physical Laboratory (Middlesex, United Kingdom) dosimeters. Variations in absorbed dose were minimized by placing thin samples (approximately 2 mm thick) within a uniform portion of the radiation field. The total mass of 5-g samples undergoing irradiation at one time during individual experiments usually did not exceed 20 g. Samples were maintained within $\pm 0.5^\circ\text{C}$ of the desired temperature by the injection of liquid nitrogen into the

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